L6 ANSWER 21 OF 21 CANCERLIT
ACCESSION NUMBER: 95607749 CANCERLIT
DOCUMENT NUMBER: 95607749
TITLE: Functional analysis of tumor suppressor genes.
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CORPORATE SOURCE: Div. of Molecular Genetics, The Netherlands Cancer Inst., Amsterdam, The Netherlands.
SOURCE: Annu Rep Neth Cancer Inst, (1993). Vol. 1993, pp. 75. ISSN: 0376-7345.
DOCUMENT TYPE: (NONGOVERNMENT REPORT)
FILE SEGMENT: ICDB
LANGUAGE: English
ENTRY MONTH: 199510

AB **Mice** nullizygous for Rb die in utero at day 12-14 of gestation and show a number of defects: abnormal liver erythropoiesis, aberrant eye lens development and extensive cell death in the nervous system. Heterozygous Rb mutant **mice** are normal but show a propensity to develop pituitary tumors. To further study the effects of loss-of-function mutations in Rb on development and tumorigenesis, we have generated chimeric **mice** using ES cells in which both alleles of Rb were inactivated via homologous **recombination**. Remarkably, Rb negative cells could contribute to most tissues of these **mice**. Rb-negative erythroid cells were indistinguishable from their wt counterparts and were found in normal proportions throughout embryonic development and adult life, indicating that Rb is not required for endstage differentiation of most cell lineages. The defect in enucleation of erythroid cells, reported previously, appears not to be a cell-autonomous phenotype of Rb-/- cells as Rb+/+ erythroid cells in chimeric embryos show a similarly retarded enucleation process. Probably, this defect is due to changes in other cell types (eg, hepatocytes). The presence of normal cells in the nervous system of chimeric embryos prevented the extensive death of Rb-negative cells, seen in homozygous Rb-/- embryos (the contribution of Rb-/- ES cells to the brain was not diminished when compared to the contribution of Rb+/+ ES cells). However, the absence of Rb protein did strongly impair the contribution of ES cells to the retina of the chimeric **mice**, indicating that Rb is required for development of a normal retina. Chimeric **mice** developed pituitary tumors with a significantly shorter latency period than **mice** bearing one functional Rb allele. Experiments are now in progress to mark the ES cell-derived cells in the embryo by LacZ or another marker so that we can study in greater detail whether certain cell lineages are missing or have been phenotypically altered. We have obtained genomic clones of p107, a close relative of Rb. We are in the process of generating targeting construct to disrupt this gene in ES cells. The aim of this program is to generate well-defined (conditional) mutations in distinct genes of the **mouse**. We apply targeted disruption of genes via homologous **recombination** in embryonic stem cells on a routine basis. In this project we are further improving this technology to circumvent some of the problems currently encountered in the field: complex pleiotropic phenotypic alterations that are difficult to interpret and embryonic lethality due to an essential role of the gene of interest during an early stage in development.

The first approach taken in this study is the introduction of conditional mutations in target genes. This is being achieved by inserting target sequences (Lox or FRT) for heterologous **recombinases** (Cre or **FLP**, respectively) on both sides of an essential exon. Tissue-specific activation or exogenous induction of the **recombinase** then results in the inactivation of the gene at a defined location/time. We have shown that both the Cre/Lox and **FLP**/FRT **recombinations** function satisfactorily in fibroblasts. FRT sites have been introduced around an essential exon in the Rb gene. At the same time we are generating a series of **transgenic mouse** strains that carry the Cre/**FLP recombinase**, either under a tissue-specific promoter or under an inducible promoter (Tet suppressor VP16). An alternative approach is the production of **transgenic mice** that carry the gene of interest directly under an inducible promoter. We are employing the Tet repressor fused to the VP16 activation domain to induce expression of the desired gene. Rb is being used to test the applicability of this approach.